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Scope of Research

Our research covers the comprehensive understanding of the physiological roles of biocatalysts (enzymes) as well as the reaction mechanism and specificity of each enzyme. **1)** Studies on diglycosidases specifically hydrolyzing the β -glycosidic bond between disaccharides and aglycons. **2)** Molecular basis of the floral aroma formation in oolong tea. **3)** Design and synthesis of transition-state analogue and mechanism-based inhibitors of γ -glutamyltranspeptidase. **4)** Design and synthesis of novel inhibitors of glycosidases and their application to affinity chromatography and biological probes to understand the physiological roles of glycosidases. **5)** Directed evolutionary studies of *Pseudomonas* lipase. **6)** Chemical knockout for probing into IAA homeostasis. **7)** Mechanism of the activation/inactivation process of plant hormones by cytochromes P450. **8)** Molecular mechanism of regulation of phenylpropanoid pathway in plants subjected to various stresses.

Research Activities (Year 2005)

Presentations

Chemical Knockouts for Probing into IAA Homeostasis – Design and Synthesis of Inhibitors of IAA-Amino Acid Conjugate Synthetases, Sakaki Y, Hiratake J, Shimizu B, Mizutani M, Sakata K, 2005 Annual Meeting of Jpn. Soc. Plant Physiologists, Niigata (Niigata), 26 March.

Directed Evolution of Lipase for Improved Amidase Activities – Saturation Mutagenesis of Substrate Binding Site – Hasegawa A, Nakagawa Y, Hiratake J, Sakata K, 2005 Annual Meeting of Kansai Branch of Jpn. Soc. Biosci. Biotech., and Agrochem., Suita (Osaka), 1 October.

Analysis of Coumarin Biosynthesis Pathway in *Arabidopsis thaliana*, Kai K, Shimizu B, Yamaguchi H, Mizutani M, Sakata K, 2005 Annual Meeting of Jpn. Soc. Chemical Regulation of Plants, 1 November.

Grants

Sakata K, Studies on Catalytic Mechanism of Disaccharide-specific Glycosidases and Evolution of Plant β -Glucosidases, Grant-in-Aid for Scientific Research (B) (2), 1 April 2004 - 31 March 2007.

Sakata K, Investigation of the Floral Aroma Formation

Controlled Affinity Purification of β -Glucosidases by Using β -Glucosylamidines as Affinity Ligand

β -Glucosylamidines are highly potent and selective inhibitors of β -glucosidases. We found that the inhibitory activity of β -glucosylamidines is attributed largely to the electrostatic interaction between the positively charged amidinium function of the inhibitor and the catalytic acid/base (Glu residue) in the enzyme active site. Hence the affinity of β -glucosylamidines can be controlled by changing the pH of the medium: β -glucosylamidines bind tightly at higher pH where the carboxy residue is deprotonated, while lower affinity results when pH is lowered below the pK_a of the carboxy group (Fig. 1a). This rationale was successfully used for the affinity purification of β -glucosidases from tea leaves. Two enzymes, β -glucosidase 1 and 2, were purified by adsorption to the affinity adsorbent (**1**) at pH 6, followed by elution at pH 4. Each enzyme was eluted sharply immediately after the pH was lowered to give the pure enzymes. The affinity of the β -glucosylamidines (ligand) towards each enzyme was highly dependent on pH: the K_i values were 0.083 and 1.9 μ M (for β -glucosidase 1) and 0.017 and 0.62 μ M (β -glucosidase 2) at pH 6 and 4, respectively. The controlled affinity purification is applicable widely to various β -glycosidases and may serve as extremely useful chemical tools to study glycosidases.

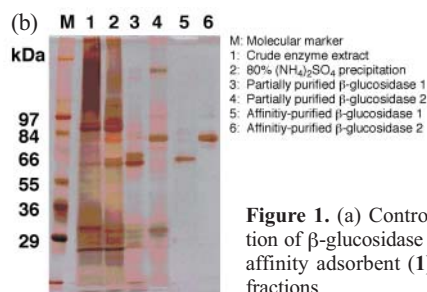
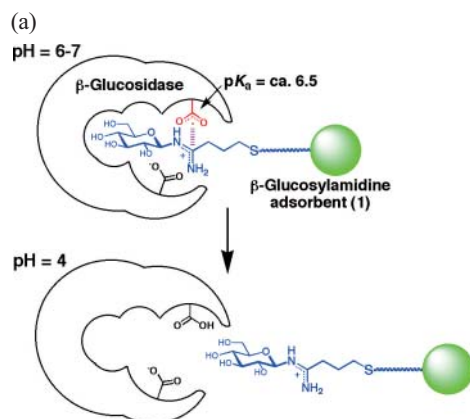


Figure 1. (a) Controlled affinity purification of β -glucosidase by β -glucosylamidines affinity adsorbent (**1**). (b) SDS-PAGE of fractions.

Characterization of P450s Involved in Steroid Hormone Biosynthesis in Plants

Brassinosteroids (BRs) are a group of plant steroids that regulate plant growth and development. Structural variation of BRs comes from the presence of several oxygen moieties at positions C-2, C-3, and C-6 in the A/B-rings and at positions C-22 and C-23 in the side chain. These oxygens are introduced into steroids by several cytochrome P450 monooxygenases (P450s). Arabidopsis *dwf4* is a brassinosteroid (BR) deficient mutant, and the *DWF4* gene encodes a P450, CYP90B1. CYP90B1 activity was measured in an *in vitro* assay, confirming that CYP90B1 is steroid C-22 hydroxylase. The substrate specificity of CYP90B1 indicated that sterols with a double bond at position C-5 are more preferred substrates than stanols, which have no double bond at the position. The results suggest that the C-22 hydroxylation of campesterol before C-5 α reduction is the main route, which contrasts with the generally accepted route via campestanol. In addition, CYP90B1 showed C-22 hydroxylation activity toward various C₂₇₋₂₉ sterols.

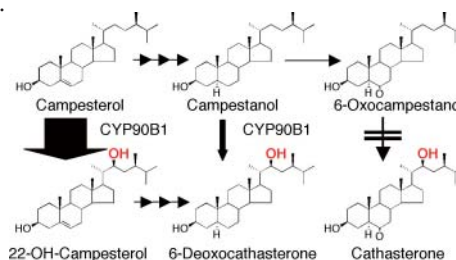


Figure 2. The main routes of the C-22 hydroxylation steps in BR biosynthesis.

Elicited by Leaf-hopper Feeding in Formosa Oolong Tea, Grant-in-Aid for Scientific Research (B) (2), 1 April 2003 - 31 March 2005.

Sakata K, Studies on Glycosidases Hydrolyzing 6-*O*-Modified β -Glucosides by Using Pseudo-sugars as Substrates, Grant-in-Aid for Exploratory Research, 1 April 2004 - 31 March 2005.

Hiratake J, Bio- and Organic Chemical Studies on Plant Glycosidases by Using β -Glycosylamidines Derivatives as Tools, Grant-in-Aid for Scientific Research (B) (2), 1 April

2004 - 31 March 2007.

Hiratake J, Chemical Tools for Probing into IAA Homeostasis - Design and Synthesis of Inhibitors of IAA-Amino Acid Conjugate Hydrolases and Synthetases, Grant-in-Aid for Exploratory Research, 1 April 2005 - 31 March 2006.

Mizutani M, Molecular Mechanisms of the Activation/Inactivation of a Plant Hormone, Grant-in-Aid for Young Scientist B, 1 April 2003 - 31 March 2005.